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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Simon C. Burton et al.	)	Group Art Unit: 1651
	)	
Application No.: 08/468,610	)	Examiner: Jon P. Weber, Ph.D.
	)	
Filed: June 6, 1995	)	Appeal No. 5415
	)	
For: CHROMATOGRAPHIC RESINS AND METHODS USING SAME	)	

BRIEF FOR APPELLANT

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Commissioner for Patents
P.O. Box 1450
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Sir:

This appeal is from the decision of the Primary Examiner dated May 5, 2003 (Paper No. 35), finally rejecting claims 1-5, 7-23, 55, and 56, which are reproduced as an Appendix to this brief.

A check covering the [ ] $165.00 (2402) [X] $330.00 (1402) Government fee and two extra copies of this brief are being filed herewith.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§1.16, 1.17, and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. A copy of this page and the signature page are submitted in duplicate.

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I. Real parties in interest

The real parties in interest are Massey University, assignee of the instant application, Genencor International, Inc., a licensee of Massey University with respect to the application, and Ciphergen Biosystems, Inc., licensee of Genencor International, Inc. with respect to technology embraced by the application.

II. Related appeals and interferences

U.S. Patent Application No. 08/468,610 is a divisional application off of U.S. Patent Application No. 08/654,937 ("'937 application"). An appeal brief for the '937 application is being submitted concurrently.

III. Status of claims

The instant application was originally filed with claims 1-23. During prosecution, claim 6 was canceled, and claims 55 and 56 were added. Accordingly, claims 1-5, 7-23, 55 and 56 are currently pending.

The status of the pending claims is as follows: Claims 1-5, 7-23, 55 and 56 stand rejected.

IV. Status of Amendments

An Amendment is being submitted concurrently with the instant Appeal Brief. Applicants request entry of amendments to claims 55-56 in the Amendment, which would obviate all outstanding rejections under 35 U.S.C. §112, first paragraph.

V. Summary of invention

The present invention is directed to a resin-protein/peptide complex. *See* p. 8, lns 10-12. The resin includes a selected ionizable ligand attached to a solid support matrix. *See* p. 7, ln 25 to p. 8, ln 9 and also see p. 22, lns 8-9. "Ionizable ligand" refers to a group containing "one or more functionalities capable of being electrostatically charged at one pH and electrostatically uncharged at another pH." *See* p. 15, lns 4-16. The term

"solid support matrix" refers to a solid resin backbone material to which a ligand can be covalently attached. *See* p. 14, lns 6-8.

Resins of the present invention are selected such that the ionizable ligand is uncharged at a pH where the target protein or peptide is bound to the resin and electrostatically charged at a pH where the target protein or peptide is desorbed from the resin. *See* p. 13, lns 1-7. An ionizable ligand is "uncharged" where "less than 5% of the ionizable functionalities on the resin are charged." *See* p. 18, lns 5-8. Accordingly, an ionizable ligand is "charged" where 5% or more of the ionizable functionalities on the resin are charged. The composition of the resins is further understood in that they "will bind about 50 percent or more of the target protein [or peptide] in the aqueous medium at low and high salt concentrations." *See* p. 28, lns 8-11.

The instant application explicitly distinguishes the resins of the present invention from polystyrene carboxyl resins (Amberlites). *See* p. 6, lns 19-27. It has been shown through experimentation that Amberlites are only uncharged at pHs less than 3. *Id.* Resins of the present invention, in contrast, are uncharged in a pH range of 5-9. *See* p. 29, lns 24-28.

VI. Issues

1) Whether claims 1-2, 4-5, 10-16, 18, 20, and 22-23 are anticipated under 35 U.S.C. §102(b) by Boardman et al. (1953).

2) Whether claims 1-5, 7-23 and 55-56 are unpatentable under 35 U.S.C. §103(a) over Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) in view of Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957) and further in view of Hancock et al (US 4,401,629), Kitamura et al. (JP 01211543), Tokuyama (JP 60137441), Kondo et al. (JP 61033130), Iimuro et al. (US 4,950,807), Bruegger (US 4,810,391), Economy et al. (US 3,835,072), and Jones et al. (US 4,154,676).

VII. Grouping of claims

1. Claims 1, 4, and 10-11 and 13-14 stand or fall together with respect to the outstanding rejection under 35 U.S.C. §102(b).

2. Claim 2 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §102(b).

3. Claim 5 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §102(b).

4. Claim 12 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §102(b).

5. Claim 15 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §102(b).

6. Claims 16, 18, 20, and 22 stand or fall together with respect to the outstanding rejection under 35 U.S.C. §102(b).

7. Claim 23 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §102(b).

8. Claims 1, 3-4, 7-11, and 13-14 stand or fall together with respect to the outstanding rejection under 35 U.S.C. §103(a).

9. Claim 2 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

10. Claim 5 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

11. Claim 12 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

12. Claim 15 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

13. Claims 16, 18,and 20-22 stand or fall together with respect to the outstanding rejection under 35 U.S.C. §103(a).

14. Claim 17 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

15. Claim 19 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

16. Claim 23 stands or falls alone with respect to the outstanding rejection under 35 U.S.C. §103(a).

17. Claims 55-56 stand or fall together with respect to the outstanding rejection under 35 U.S.C. §103(a).

VIII. Argument

Applicants are concurrently filing an Amendment that would obviate all outstanding rejections under 35 U.S.C. §112, first paragraph, with the instant Appeal Brief. Applicants accordingly are not presenting arguments regarding the noted rejection.

A. Rejection of claims 1, 4, 10-11 and 13-14 under 35 U.S.C. §102(b).

1. The Rejection

Claims 1, 4, 10-11 and 13-14 stand rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953).

2. Anticipation Under 35 U.S.C. §102

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP citing *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

3. Elements of Claim 1

Applicants address the anticipation rejection below through a discussion of independent claim 1, which reads as follows:

> 1. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises
> a) a solid support matrix; and
> b) selected ionizable ligand covalently attached to the matrix
> wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at a high and a low ionic strength at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH and further wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength.

Several elements are present in claim 24, but three will be highlighted in view of the

outstanding rejection. First, the claim recites that the resin is electrostatically uncharged at the pH where the target protein or peptide is resin bound, regardless of whether the medium is at a high or a low ionic strength. In other words, less than 5% of the resin's ionizable functional groups are ionized at a high ionic strength at a binding pH; and, less than 5% of the resin's ionizable functional groups are ionized at a low ionic strength at a binding pH. The instant application defines "high ionic strength" on page 18, lines 18-21: "The term 'high ionic strength' means an ionic strength greater than or equal to that required to provide a conductivity of 4.7 millimho (milliSeimens (mS/cm^2)). For example, such conductivity can be reached by using 250 millimolar (mM) sodium chloride)."

Second, the claim recites that the resin includes an ionizable ligand covalently attached to a solid support matrix. The instant application defines such a ligand-matrix arrangement through reference to equations included on page 16:

> As noted above, the ionizable and non-ionizable ligands are either directly and covalently linked to the solid support matrix or these ligands comprise a spacer arm for covalently linking the ionizable functionality to the solid support matrix. Accordingly, an attachment of an ionizable ligand to the solid support matrix can be illustrated as follows:
>
> Solid Support Matrix-Ionizable Ligand
>
> Insofar as the ligand can comprise a spacer arm, the above formula can be further illustrated as:
>
> Solid Support Matrix-$[\text{Spacer Arm}]_n$-R
>
> Where n is 0 or 1; R is an ionizable functionality and Spacer Arm is a chemical group capable of covalently linking the ionizable functionality to the solid support matrix.

In short, the ionizable ligand is separate from, and not part of, the solid support matrix. It is something attached to the solid support matrix through a chemical transformation.

Third, the claim recites that about 50 percent or more of the target protein/peptide binds to the resin regardless of whether the aqueous medium is at high or low ionic strength. This statement points to the salt-independent nature of the complex: Whether the sodium concentration of the aqueous medium is 2 mM or 500 mM the complex remains essentially intact.

4. Boardman et al.

Boardman *et al.* (hereinafter "Boardman") is a 1953 Nature article cited against the currently claimed invention. The article discusses a study designed to find an optimal pH for the chromatographic purification of hemoglobins using an Amberlite resin—IRC 50. Boardman reports that the separation is conducted at a single pH (*e.g.*, 5.8) and that the yields of pure components are low. *Boardman*, p. 210.

a. Boardman's resin is not "uncharged."

Figure 1(a) of Boardman is a graph showing both elution profiles and titration profiles. As can be seen, cytochrome c elution, or non-binding, at two distinct ionic strengths begins at a pH about 6 or higher. The sodium ion uptake during titration in the higher ionic strength medium at pH 6 is approximately 50% from the graph, while it is approximately 20% at the lower ionic strength. According to applicants' definition of "uncharged," therefore, Boardman only reports a resin that is electrostatically charged at a pH where cytochrome c is resin bound, regardless of the medium's ionic strength. Such resin properties are clearly outside the scope of applicants' presently claimed invention.

Furthermore, applicants previously submitted a Declaration under 37 C.F.R. § 1.132 by Nathaniel T. Becker. (See Appendix B) The Becker declaration discusses the Amberlite IRC-50 resin utilized in Boardman. It further explains product literature for the resin as provided by the manufacturer, Rohm and Haas, Co. (2000) ("Rohm and Haas"). A copy of the Rohm and Haas product literature accompanies the attached copy of the Becker declaration.

In particular, as set forth in the declaration, Amberlite IRC-50 is a weakly acidic cation exchange resin that has a pK value of 6.1, meaning it is still 50 percent charged at a pH of 6.1. The resin is weakly acidic and retains a partial charge at pH 5, becoming fully protonated (neutralized) only at a pH of between 2.5 and 4.0. This notion is further supported by Figure 3 of the Rohm and Haas product literature. The point of zero net charge is equivalent to the pH at which zero milliequivalents of base (KOH) has been applied to the resin, which is represented by the position where the titration curves intersect the y-axis (pH) at zero on the x-axis (mEq KOH). This value will vary slightly depending on the buffer salts, but is at most a pH of 4.0 for pure water. Accordingly, the Becker

declaration highlights the manufacturer's clear indication that Boardman's resin remains charged at the pH where it binds the protein (*i.e.*, pH of 5 to 9).

Finally, both ionic strengths at which Boardman reports elution profiles are low according to applicants' definition. The legend of Figure 1 clearly states that the lower ionic strength medium contains 0.17 gm sodium ions/L, which corresponds to a sodium ion concentration of about 7.5 mM; the higher ionic strength medium contains 0.34 gm sodium ions/L, which corresponds to a sodium ion concentration of about 15 mM. Applicants, as noted above, define "high ionic strength" to be a sodium ion concentration of about 250 mM or above. In other words, the highest ionic strength at which Boardman works is approximately 17 times lower than applicants' "high ionic strength" medium. Clearly then, Boardman teaches nothing regarding resin-protein/peptide complexes at a high ionic strength.

b. Amberlite resin does not contain ionizable ligands.

Amberlites, including IRC 50, are polystyrene carboxyl resins. The ionizable functionality on the resin is a carboxylic acid group, which is incorporated into the resin when it is synthesized. In other words, the ionizable group of IRC 50 is part of the resin's solid support matrix; it is not covalently attached to the matrix through a chemical transformation. This is in direct contrast to applicants' presently claimed invention, which requires that the resin's ionizable functionality be covalently attached to the solid support.

c. Amberlite resin complexes are not salt independent.

Amberlite is an ion exchange resin. A common method used to elute (*i.e.*, desorb) proteins from an ion exchange resin involves increasing the ionic strength of an elution medium while keeping the pH constant. In other words, one can desorb a protein simply by adjusting a medium's ionic strength from low to high. This is distinguished from applicants' invention, which is directed to a complex that is essentially maintained regardless of a medium's ionic strength.

5. Boardman does not anticipate the subject claims.

The arguments provided above for claim 1 equally apply to claims 4, 10-11 and 13-14. Boardman does not provide all the elements of listed claims and therefore does not anticipate them.

B. Rejection of claim 2 under 35 U.S.C. §102(b).

Claim 2 stands rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953). The arguments provided in section VIII(A) above apply to claim 2. Furthermore, the subject claim states that the resin's ionizable ligand "is positively charged at the pH where the target protein or peptide is desorbed from the resin." This element further distinguishes claim 2 from Boardman in that Amberlite IRC-50—the resin of Boardman—contains groups (*i.e.*, carboxylic acid moieties) that can only be neutral or negatively charged.

C. Rejection of claim 5 under 35 U.S.C. §102(b).

Claim 5 stands rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953). The arguments provided in section VIII(A) above apply to claim 5. Furthermore, the subject claim states that "the ionizable ligand comprises a spacer arm . . . wherein the ionizable functionality is attached to the solid support matrix via the spacer arm." This element further distinguishes claim 5 from Boardman in that Amberlite IRC-50 does not contain any moiety that can fairly be characterized as a "spacer arm."

D. Rejection of claim 12 under 35 U.S.C. §102(b).

Claim 12 stands rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953). The arguments provided in section VIII(A) above apply to claim 12. Furthermore, the subject claim states that "the solid support matrix is non-ionizable." This element further distinguishes claim 12 from Boardman in that the carboxylic acid groups on Amberlite IRC-50 are part of the solid support matrix.

E. Rejection of claim 15 under 35 U.S.C. §102(b).

Claim 15 stands rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953). The arguments provided in section VIII(A) above apply to claim 15. Furthermore, the subject claim states that "the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the opposite polarity from the net electrostatic charge on the target protein or peptide at the pH of desorption." This element further distinguishes claim 15 from Boardman in that cytochrome c, as reported, is desorbed at a pH where both Amberlite IRC-50 and cytochrome c have net negative charges.

F. Rejection of claims 16, 18, 20, and 22 under 35 U.S.C. §102(b).

1. The Rejection

Claims 16, 18, 20, and 22-23 stand rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953).

2. The Argument

Independent claim 16 is similar to claim 1 discussed above, with at least one exception: the resin's ionizable functionality is incorporated into the backbone of the solid support matrix. Applicants' arguments regarding the charged nature of Boardman's resin and the salt independent character of the complex, however, apply to the subject claims. Claims 16, 18, 20, and 22-23 are accordingly not anticipated by Boardman for that reason.

G. Rejection of claim 23 under 35 U.S.C. §102(b).

Claim 23 stands rejected under 35 U.S.C. §102(b) as being anticipated by Boardman et al. (1953). The arguments provided in section VIII(F) above apply to claim 23. Furthermore, the subject claim states that "the electrostatic charge induced on the resin-protein complex is of the opposite polarity from the net electrostatic charge on the target protein or peptide at the pH of desorption." This element further distinguishes claim 23 from Boardman in that cytochrome c, as reported, is desorbed at a pH where both Amberlite IRC-50 and cytochrome c have net negative charges.

H. Rejection of claims 1, 3-4, 7-11, and 13-14 under 35 U.S.C. §103(a).

1. The Rejection

Claims 1, 3-4, 7-11 and 13-14 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) in view of Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957) and further in view of Hancock et al. (US 4,401,629), Kitamura et al. (JP 01211543), Tokuyama (JP 60137441), Kondo et al. (JP 61033130), Iimuro et al. (US 4,950,807), Bruegger (US 4,810,391), Economy et al. (US 3,835,072), and Jones et al. (US 4,154,676).

2. Prima Facie Obviousness under 35 U.S.C. §103

"To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable

expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." MPEP 2143. "The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure." MPEP citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

3. Sasaki et al. (1979) and Sasaki et al. (1981) have significant deficiencies.

Sasaki (1979) and Sasaki (1981) are both concerned with a purification method denoted as "hydrophobic-ionic chromatography." Both references report the use of Amberlite CG-50 resin, a resin containing carboxylic acids, which is almost identical in structure to that used by Boardman (*i.e.*, IRC-50). Sasaki (1979) reports that porcine pancreas enzymes are adsorbed onto CG-50 at a pH less than 4.5, preferably at pH 4.0, and chromatographically purified. This pH appears to be critical to Sasaki (1979), as the subject proteins are not stable at neutral or basic pHs. See Sasaki (1979), p. 1538.

Sasaki (1981) reports the application of hydrophobic-ionic chromatography to other acid stable enzymes, including microbial glucose oxidase, hyaluronidase, cholesterol oxidase, and cholesterol esterase. The reference explicitly states that a low pH is required for the discussed purification method: "[H]ydrophobic-ionic chromatography with Amberlite CG-50 has the disadvantage that a pH as acidic as 4.5 is required in the process of adsorption." Sasaki (1981), p. 1561. The reference further indicates that hydrophobic-ionic chromatography is only suitable for the purification of acid-stable compounds. *Id.* and see Abstract.

Applicants' subject claims do not read on either Sasaki article, and the examiner has not asserted that they do. There are obvious, significant differences between the presently claimed invention and the process of Sasaki, but only two will be mentioned here: Hydrophobic-ionic chromatography necessarily requires an enzyme to be bound to a resin at a pH of 4.5 or less. Applicants' complex, in contrast, requires that the binding event occur at a pH of 5 to 9. Furthermore, applicants' presently claimed invention requires the use of a resin having ionizable functionality that is not part of its backbone.

4. There is no motivation to modify Boardman or either Sasaki reference to arrive at applicants' presently claimed invention.

a. Boardman's Amberlite resin affords low yields.

Boardman reports the following regarding the suitability of his hemoglobin purification process:

"While the separation of the two haemoglobins [sic] described above is satisfactory for some purposes, and could not doubt be improved somewhat by the use of a rather long column, the yields of the pure components were low. It appears probable that, if the material in peak C has indeed been denatured by passage down the column, yields could be improved by working at lower temperatures. A study of the nature of the processes leading to changes in the protein on the surface of the resin is in progress, in hope that extension of the procedure to the separation of other protein systems will be possible." Boardman, p. 210.

i. Boardman's process employing an Amberlite resin is seriously flawed.

By Boardman's own admission, his purification process cannot provide even moderate yields of pure hemoglobins. The reference further states that the process may be suitable for "some purposes," but there is no elaboration as to what those purposes would be.

ii. Boardman's process is limited to the examined hemoglobins.

Boardman indicates that proteins change on the surface of IRC-50. The noted changes lead to undesirable results, and Boardman fails to suggest known, reliable methods to prevent such results.

iii. Boardman's suggestions regarding improvement have nothing to do with applicants' presently claimed invention.

Boardman only makes two suggestions for improving the reported process: 1) lowering the temperature; and, 2) lengthening the chromatography column. Neither suggestion is related to applicants' complex. In other words, Boardman does not suggest improving his process through the use of a resin that is electrostatically uncharged at binding pH, and that has covalently attached ionizable functionality.

iv. The Sasaki references are limited to acid-stable proteins/ peptides.

At best, Sasaki suggests that Boardman can be improved by focusing on acid-stable enzymes. The Sasaki references report moderate to high yields of purified enzymes by conducting an adsorption step at pH 4.5 or below on an Amberlite resin. Boardman and the Sasakis taken together, then, suggest that protein changes on an Amberlite resin can be controlled by forming a resin-enzyme complex at a pH of 4.5 or below. The protein/peptide complexes of applicants' presently claimed invention, however, are formed at a pH of 5 to 9—a range that would denature the enzymes investigated in Sasaki (1979) and Sasaki (1981).

b. The other cited references do not suggest modifying Boardman or the Sasakis to arrive at applicants' invention.

The examiner cites many different references in support of the subject 35 U.S.C. §103 rejection. The cited references with summaries are as follows:

Kunin (1958)—Description of cation exchange resin characteristics, primarily directed to amberlites.

Topp (1949)—Properties of ion exchange resins such as sulphonated, crosslinked polystyrene.

Kitchener (1957)—Effect of pH on resin exchange equilibria.

Guthrie (1957)—Ion exchange properties of chemically modified cotton fabrics.

Hancock (US 4,401,629)—A method of making a polymeric ion exchange resin containing imidazole residues.

Kitamura (JP 01211543)—A method of making a bisphenol polymer involving the condensation of phenol and acetone.

Tokuyama (JP 60137441)—A method of making an ion exchange resin containing a carboxyl group, a sulfonic acid group and a phenolic hydroxy group.

Kondo (JP 61033130)—A method of making a 2-haloacrylic acid and related derivates.

Iimuro (US 4,950,807)—A method of making a bisphenol polymer involving the condensation of phenol and acetone.

Bruegger (US 4,810,391)—Separation of hemoglobin A_2 from hemoglobin mixture using resins containing groups such as carboxylic acid groups (e.g., Amberlite).

Economy (US 3,835,072)—A method of making a cured infusible phenolic resin.

Jones (US 4,154,676)—Ion exchange processes using regenerated cellulose based processes.

As can be seen, the references are primarily directed to methods of making ion exchange resins; a few are directed to physical properties of well-known resins. Only Bruegger discusses the addition of a protein to a resin, and the discussion refers to the use of Boardman and Sasaki-like resins (*i.e.*, Amberlites). None of the references suggests how one of ordinary skill could modify the Amberlite resins of Boardman and the Sasakis to arrive at applicants' resin-protein/peptide complex.

5. There was no reasonable expectation of success.

a. Boardman points to existing problems.

As discussed above, Boardman notes that proteins change when they interact with Amberlite resins. The change results in low yields of purified hemoglobins upon protein elution.

b. The Sasakis suggest an acid-focused process.

Both Sasaki references point to the fact that, to purify an enzyme using hydrophobic-ionic chromatography, enzyme adsorption on an Amberlite resin must occur at pH 4.5 or below.

c. The other cited references do not teach principles directed to controlling such interactions.

The cited references do not teach principles by which one could prevent the deleterious interactions of proteins or peptides with resins. Accordingly, one could not

know *a priori* whether the resins used by applicants', which bind proteins/peptides at a pH of 5 to 9, would be useful or destructive in the formation of a resin-protein/peptide complex.

d. Without guiding principles, no one could predict success.

In the absence of principles that would guide one to modify the Amberlite resins of Boardman and the Sasakis, one of ordinary skill could not have predicted that any particular modification would have resulted in a viable resin-protein/peptide complex.

I. Rejection of claim 2 under 35 U.S.C. §103(a).

Claim 2 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the same reasons as claim 1. *See* section VIII(H)(1) above. The arguments provided in section VIII(H) above apply to claim 2. Furthermore, the subject claim is further distinguished from the cited references as noted in section VIII(B) above.

J. Rejection of claim 5 under 35 U.S.C. §103(a).

Claim 5 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the same reasons as claim 1. *See* section VIII(H)(1) above. The arguments provided in section VIII(H) above apply to claim 5. Furthermore, the subject claim is further distinguished from the cited references as noted in section VIII(C) above.

K. Rejection of claim 12 under 35 U.S.C. §103(a).

Claim 12 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the same reasons as claim 1. *See* section VIII(H)(1) above. The arguments provided in section VIII(H) above apply to claim 12. Furthermore, the subject claim is further distinguished from the cited references as noted in section VIII(D) above.

L. Rejection of claim 15 under 35 U.S.C. §103(a).

Claim 15 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the same reasons as claim 1. *See* section VIII(H)(1) above. The arguments provided in section VIII(H) above apply to claim 15. Furthermore, the subject claim is further distinguished from the cited references as noted in section VIII(E) above.

M. Rejection of claims 16, 18, and 20-22 under 35 U.S.C. §103(a).

1. The rejection

Claims 16, 18, and 20-22 stand rejected under 35 U.S.C. §103(a) as being

unpatentable over Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) in view of Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957) and further in view of Hancock et al. (US 4,401,629), Kitamura et al. (JP 01211543), Tokuyama (JP 60137441), Kondo et al. (JP 61033130), Iimuro et al. (US 4,950,807), Bruegger (US 4,810,391), Economy et al. (US 3,835,072), and Jones et al. (US 4,154,676).

2. The argument

As noted in section VIII(F) above, independent claim 16 is similar to claim 1 except for the attachment mode of the employed resin's ionizable functionality. Arguments regarding motivation to combine and predictability of success presented in section VIII(H) above apply equally to the instant claims despite that difference. Accordingly, one of ordinary skill would not have been motivated to modify Boardman or the Sasakis to arrive at the presently claimed invention.

N. Rejection of claim 17 under 35 U.S.C. §103(a).

Claim 17 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the same reasons as claim 16. See section VIII(M)(1) above. The arguments provided in section VIII(M)(2) above apply to claim 17. Furthermore, the subject claim states that "the ionizable functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is positively charged at the pH where the target protein or peptide is desorbed from the resin." This element further distinguishes claim 17 from the cited references in that desorption occurs when the resin possesses a positive charge rather than a negative charge. *See* Boardman's discussion regarding cytochrome c.

O. Rejection of claim 19 under 35 U.S.C. §103(a).

Claim 19 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the same reasons as claim 16. See section VIII(M)(1) above. The arguments provided in section VIII(M)(2) above apply to claim 19. Furthermore, the subject claim states that "the ionizable functionality comprises amino groups covalently attached in the backbone of the solid support matrix." This element further distinguishes claim 19 from the cited references, as they do not disclose such a resin.

P. Rejection of claim 23 under 35 U.S.C. §103(a).

Claim 23 stands rejected under 35 U.S.C. §103(a) as being unpatentable for the

same reasons as claim 16. See section VIII(M)(1) above. The arguments provided in section VIII(M)(2) above apply to claim 23. Furthermore, the subject claim states that "the ionizable functionality comprises amino groups covalently attached in the backbone of the solid support matrix." Furthermore, the subject claim is further distinguished from the cited references as noted in section VIII(G) above.

Q. Rejection of claims 55-56 under 35 U.S.C. §103(a).

1. The rejection

Claims 55-56 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Boardman et al. (1953), Sasaki et al. (1979) and Sasaki et al. (1982) in view of Kunin (1958), Topp et al. (1949), Kitchener (1957) and Guthrie (1957) and further in view of Hancock et al. (US 4,401,629), Kitamura et al. (JP 01211543), Tokuyama (JP 60137441), Kondo et al. (JP 61033130), Iimuro et al. (US 4,950,807), Bruegger (US 4,810,391), Economy et al. (US 3,835,072), and Jones et al. (US 4,154,676).

2. The argument

Independent claim 55 is similar to claim 1 except that the selected ionizable ligands are specified: amine groups, phenolic groups, histidyl groups, pyridyl groups, anilino groups, morpholinyl groups, and imidazoyl groups. Claim 56 is similar to claim 16 with the same exception.

a. There is no motivation to modify Boardman or the Sasakis to arrive at applicants' invention.

Applicants have pointed out that one of ordinary skill in the art would not have been motivated to modify Boardman's problematic method or the Sasakis' Amberlite resins to arrive at applicants' invention as recited in claim 1. *See* section VIII(H) above. This argument is especially true where the ionizable ligands are specifically listed as in claims 55 and 56. If one does not understand how to modify an Amberlite resin containing ionizable carboxylic acid groups to provide a viable resin-protein/peptide complex, one certainly does not understand that using an amine-functionalized resin would do the trick.

b. One could not have predicted that the specified resins would have yielded successful results.

As in section VIII(H) above, if one does not understand principles that would allow him to predict whether a resin could be successfully used to form a resin-protein/ peptide

complex, one cannot predict whether a particular, modified resin (*e.g.*, amine modified or phenol modified) will work.

IX. Conclusion

For the reasons present above, applicants' submit that the rejection of claims 1-5, 7-23 and 55-56 is in error. Reversal of the rejections is respectfully requested.

Respectfully submitted,

BURNS DOANE SWECKER & MATHIS LLP

By: _____

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APPENDIX A

1. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises

a) a solid support matrix; and

b) selected ionizable ligand covalently attached to the matrix

wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at a high and a low ionic strength at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH and further wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength.

2. The resin-protein complex of Claim 1 wherein the ionizable ligand is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is positively charged at the pH where the target protein or peptide is desorbed from the resin.

3. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is negatively charged at the pH where the target protein or peptide is desorbed from the resin.

4. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand comprises an ionizable functional group directly attached to the solid support matrix.

5. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand comprises a spacer arm and at least one ionizable functionality wherein the ionizable functionality is attached to the solid support matrix via the spacer arm.

7. The resin-protein/peptide complex of Claim 1 wherein the resin further comprises non-ionizable ligands.

8. The resin-protein/peptide complex of Claim 7 wherein the percentage of non-ionizable ligands attached to the solid support matrix based on the total of ionizable and non-ionizable ligands ranges from greater than 0% to about 80%.

9. The resin-protein/peptide complex of Claim 8 wherein the percentage of non-ionizable ligands attached to the solid support matrix based on the total of ionizable and non-ionizable ligands ranges from greater than 0% to about 40%.

10. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix is cross-linked.

11. The resin-protein/peptide complex of Claim 1 wherein the resin contains from about 0.05 mmol to about 0.5 mmol ionizable ligand per ml of the solid support matrix prior to covalent attachment of any non-ionizable ligand.

12. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix is non-ionizable.

13. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix contains ionizable functionality which functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin.

14. The resin-protein/peptide complex of Claim 1 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the same polarity as the net electrostatic charge on the target protein or peptide at the pH of desorption.

15. The resin-protein/peptide complex of Claim 1 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the opposite polarity

from the net electrostatic charge on the target protein or peptide at the pH of desorption.

16. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises

a) a solid support matrix having a selected ionizable functionality incorporated into the backbone thereof wherein the ionizable functionality is selected such that the resin is electrostatically uncharged at a high and a low ionic strength at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH; and

b) optionally a non-ionizable ligand covalently attached thereto,

wherein about 50 percent of more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength.

17. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is positively charged at the pH where the target protein or peptide is desorbed from the resin.

18. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is negatively charged at the pH where the target protein or peptide is desorbed from the resin.

19. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality comprises amino groups covalently attached in the backbone of the solid support matrix.

20. The resin-protein/peptide complex of Claim 16 wherein the solid support matrix is cross-linked.

21. The resin-protein/peptide complex of Claim 16 wherein the resin contains from about 0.05 mmol non-ionizable ligand per ml of the solid support matrix.

22. The resin-protein/peptide complex of Claim 16 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the same polarity as the net electrostatic charge on the target protein or peptide at the pH of desorption.

23. The resin-protein/peptide complex of Claim 16 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the opposite polarity from the net electrostatic charge on the target protein or peptide at the pH of desorption.

55. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises

a) a solid support matrix; and

b) selected ionizable ligand covalently attached to the matrix,

wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at a high and a low ionic strength at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH, and wherein said ionizable ligand is selected from group consisting of amine groups, phenolic groups, histidyl groups, pyridyl groups, anilino groups, morpholinyl groups, and imidazolyl groups, and further wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength.

56. A resin-protein/peptide complex which comprises a resin and a target protein

or peptide bound thereto wherein said resin comprises

a) a solid support matrix having a selected ionizable functionality incorporated into the backbone thereof wherein the ionizable functionality is selected such that the resin is electrostatically uncharged at a high and a low ionic strength at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin, wherein desorption occurs by a change in the pH from the binding pH, and further wherein said ionizable ligand is selected from group consisting of amine groups, phenolic groups, histidyl groups, hydroxyl groups, pyridyl groups, anilino groups, morpholinyl groups, and imidazolyl groups; and

b) optionally a non-ionizable ligand covalently attached thereto,

wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength.

APPENDIX B

Patent Application
Attorney's Docket No. 010055-134

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)
	)
Simon C. BURTON et al.	) Group Art Unit: 1651
	)
Application No.: 08/468,610	) Examiner: Jon P. Weber, Ph.D.
	)
Filed: June 6, 1995	)
	)
For: CHROMATOGRAPHIC RESINS AND METHODS FOR USING SAME	)

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Nathaniel T. BECKER, do hereby declare:

1. THAT, I have received a Bachelor of Science Degree in chemical Engineering from Stanford University in 1983, and a Master of Science Degree from the University of California, Davis in 1984.

2. THAT, I am an employee of Genencor International, Inc. (hereinafter "Genencor"), where I have worked since 1985. I am a biochemical engineer by training and am currently the Group Leader for the Delivery Systems group at Genencor. I spent more than eight of my seventeen years at Genencor developing separation and purification processes for the production of enzymes and proteins at industrial scale. This includes extensive experience developing and inventing processes and techniques based on the following technologies: centrifugation, filtration, extraction, chromatography (ion exchange, hydrophobic, affinity), cross-flow membrane filtration,

crystallization and precipitation. I have numerous patent applications and several issued patents in the areas of protein crystallization and chromatography. Most specifically, I have carried out and directed experimental work involving the separation of enzymes and proteins using ion exchange chromatography and other forms of chromatography, which requires a knowledge of how to prepare, adsorb and elute proteins from ion exchange columns.

3. THAT, a copy of my Curriculum Vitae is attached hereto as Appendix A.

4. THAT, I am one of the inventors of the subject matter disclosed and claimed in the above-referenced application, and I have reviewed and am familiar with the contents of U.S. Patent Application Serial No. 08/468,610 (hereinafter "'610 patent application") including currently pending claims 1-5 and 7-23.

5. THAT, the invention in the '610 patent application relates to complexes of chromatographic resins with proteins and peptides. In particular, the chromatographic resins are useful for the binding of a target protein or peptide from an aqueous medium. Central to the claimed invention is the use of an electrostatically uncharged resin at the pH where the target protein or peptide is bound to the resin which has a pH in the range of from 5 to 9. In addition, the resin is selected such that it contains an electrostatic charge at the pH where the protein or peptide is desorbed from the resin, wherein the desorption occurs by a change in the pH from the binding pH.

6. THAT, I have reviewed and am familiar with the Office Action dated September 28, 2001. I have also reviewed and am familiar with the Examiner's rejection of the claims alleging that such claims are purportedly anticipated by Boardman et al., *Nature*, 171:208-210 (1953) (hereinafter "Boardman").

7. THAT, I have reviewed and am familiar with the product literature regarding the synthetic cation exchange resin, Amberlite IRC-50, provided by the manufacturer, Rohm and Haas Co. (2000) (hereinafter "Rohm and Haas"). A copy of the Rohm and Haas product literature is attached hereto as Appendix B.

8. THAT, I have reviewed and am familiar with the Boardman reference. In relation to this reference, the September 28, 2001, Office Action contains the following statement:

> "At a low pH the cation exchange media is uncharged and binds the proteins. As the pH is raised, the protein is eluted. Figure 1(a) illustrates the technique with cytochrome C on Amberlite IRC-50 [a cross-linked poly(methacrylic acid) with a capacity of 10 Meq/g]. At a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged."

9. THAT, in my opinion the above statement regarding the Amberlite IRC-50 resin is inaccurate. By definition, and as is recognized by those skilled in the art, Amberlite IRC-50 is a weakly acidic cation exchange resin based on macroreticular methacrylic acid-divinylbenzene chemistry. It has a pK value of 6.1, meaning that it is still 50% charged at pH 6.1 The charged moiety on the resin is a carboxylic acid group within the methacrylic acid functionality. Given that the resin is weakly acidic, it retains a partial charge at pH 5, and becomes fully protonated (neutralized) only at a pH of between 2.5 and 4.0, depending on the buffer salts present. This is clearly demonstrated in the product literature provided by the manufacturer, Rohm and Haas. According to Rohm and Haas, the point of zero net charge is equivalent to the pH at which zero millequivalents of base (KOH) have been applied to the resin, which is represented by where the titration curves intersect the y-axis (pH) at zero on the x-axis (mEq KOH) (see Figure 3). This will vary slightly depending on the buffer salts, but, at most, is pH 4.0 for pure water. Thus, it is

my opinion that the Amberlite IRC-50 cation exchange resin in Boardman remains charged at the pH where it binds the protein.

10. THAT, in my opinion those skilled in the art would recognize that no basis is seen in the Office Action or in Boardman to conclude that Boardman teaches binding a protein to an uncharged resin in the range of pH 5 to 9. Accordingly, the Amberlite IRC-50 cation exchange resin of Boardman fails to disclose the claimed invention. Thus, the Boardman reference neither anticipates nor renders obvious the claimed invention.

11. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 1-25-02 Signed: Nathaniel T. Becker

Nathaniel T. Becker

APPENDIX A

Nathaniel Todd Becker
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Email: tkbecker@yahoo.com

EDUCATION

Brown University, A.B., Philosophy (magna cum laude), 1979
Stanford University, B.S., Chemical Engineering, 1983 and M.A., Philosophy, 1983
University of California, Davis, M.S., 1984

EMPLOYMENT HISTORY

Genentech, Inc., South San Francisco, Ca.

1983- 1984 Biochemical engineering intern

Genencor International, Inc., Palo Alto, Ca.

1985-1989 Advanced from research associate to scientist in the area of enzyme recovery and purfication. These responsibilities included technical responsibility for developing and scaling up downstream processes based on centrifugation, filtration, membrane separations, chromatography and crystallization

1989-2001 Advanced from scientist to staff scientist, currently Group Leader for the Delivery Systems group. Responsibilities included development and commercial deployment of liquid and solid formulation technologies, including stabilization, granulation and coating, and controlled release technology. This has included project leader responsiblities for over ten new products, and inventorship on more than a dozen patents and patent applications in the area of purification, formulation and granulation of enzymes.

PUBLICATIONS (Selected)

Becker, T., Park, G. and Gaertner, A.L. (1997). Formulation of detergent enzymes. In: Van Ee, J.H., Misser, O. and Baas, E. (eds). Enzymes in Detergency. Marcel Dekker, New York, pp. 299-325.

Becker, T. (1995). Separation and Purification Processes for the Recovery of Industrial Enzymes, Ch. 14 in Bioseparation Processes in Foods, R.K. Singh and S.S. H. Rizvi, eds., Marcel Dekker, New York, 1995, pp. 427-445.

APPENDIX B

AMBERLITE® IRC50

Synthetic Cation Exchange Resin

ROHM HAAS

PRODUCT DATASHEET

AMBERLITE IRC50 is a synthetic cation exchange resin produced in the form of white, opaque beads. Its unusually high exchange capacity is derived from carboxylic acid groups. Supplied in the hydrogen or "free-acid" form, AMBERLITE IRC50 can be converted readily to the sodium salt by treatment with a solution of sodium hydroxide. In the sodium form, the resin undergoes reactions typical of the salt of a weak acid and strong base.
Because of its selectivity for the hydrogen ion, any adsorbed cation can be desorbed easily with a regeneration efficiency approaching 100% by treatment with dilute mineral acid. The carboxylic functionality and exchange selectivities of AMBERLITE IRC50 lead to immediate consideration of this ion exchange resin in a variety of applications such as the neutralisation of strong bases; the recovery of metallicions; the isolation and concentration of antibiotics; basic amino acids, enzymes and peptides.

PROPERTIES

Physical form	White opaque beads
Ionic form as shipped	H^+
Total exchange capacity [1]	≥3.0 eq/L (H^+ form)
Moisture holding capacity [1]	43 to 53% (H^+ form)
Shipping weight	660 g/L
Particle size	
Harmonic mean size [1]	280-700 µm
Uniformity coefficient [1]	≤2.0
Fines content [1]	<0.300 mm: 8.0% max
Coarse beads [1]	>1.180 mm: 5.0% max
Maximum reversible swelling	$H^+ \rightarrow Na^+$: 100% $H^+ \rightarrow Ca^+$: 40%

[1] *Contractual value*

SUGGESTED OPERATING CONDITIONS

Maximum operating temperature	100°C	
Minimum bed depth	600 mm	
Service flow rate	8 to 16 BV*/h	
Regenerants	HCl or H_2SO_4	
Concentration (%)	2 to 5	0.5 to 0.7
Flow rate (BV/h)	2 to 8	15 to 40
Level	See text	
Rinse water requirements	4 to 7 BV	
Service flow rate	8 to 16 BV*/h	

**BV (Bed Volume) = 1 m^3 solution per m^3 resin*

PHYSICALSTABILITY

EffectofTemperature

The rate of exchange and the affinity for hydrogen (H^+) increase as operating temperaturesareelevated

Attrition

Extended field experience has shown that AMBERLITE IRC50 has excellent attrition resistance.

CHEMICALSTABILITY

AMBERLITEIRC50isstableinthepresenceof strongalkalisandacids,aliphaticandaromatic solvents. On prolonged contact with certain organic solvents, the resin swells to some extent,butnodisintegrationoftheexchanger beadshasbeenobserved.

OPERATIONALCHARACTERISTICS

PressureDrop

The approximate drop in pressure to be expected for each metre of bed depth of AMBERLITE IRC50 in normal downflow operation at various flow rates and temperaturesisindicatedbythedatainFigure 1.

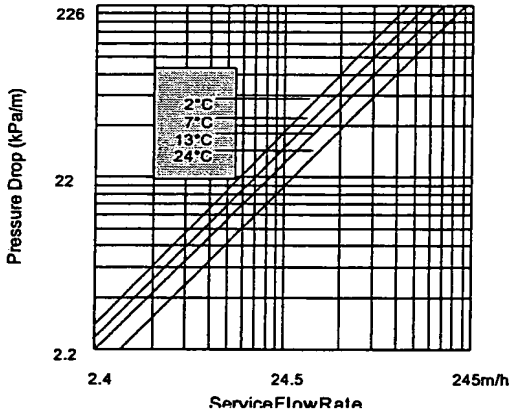

Figure I

HydraulicExpansion

To ensure proper cleaning and hydraulic classificationofAMBERLITEIRC50aftereach operational cycle, the bed of resin should be backwashedwithwaterforabouttenminutesat a flow rate sufficient to effect a minimum of 50%expansioninbedvolume.

The hydraulic expansion of the bed during backwashing operations is reported as a function of the flow rate at various temperaturesinFigures2aand2b.

Values for the calcium and sodium forms are usedintheexample.

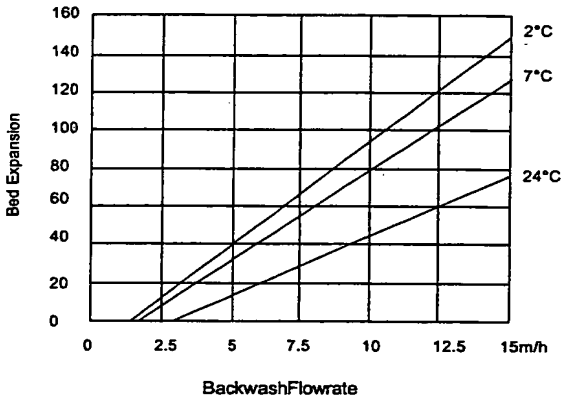

Figure2a: HydraulicExpansion(Ca)

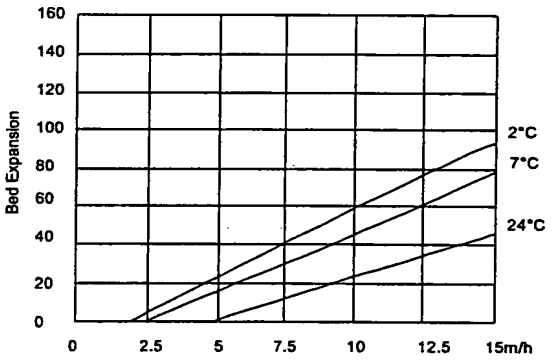

Figure2b: Hydraulicexpansion(Na)

EXCHANGECAPACITY

The total exchange capacity of AMBERLITE IRC50 is attainable only at high pH values. In strongly alkaline media, it is possible to utilise all of the carboxylic acid groups calculated to be present in the resin matrix.

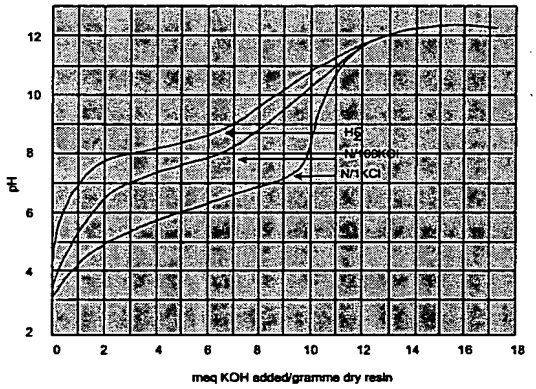

Figure3: Titration curves

ORGANICCOMPOUNDS

The buffering capacity and acid-elution efficiency of AMBERLITE IRC50 have been demonstrated by applying the exchanger to the adsorption and recovery of basic amino acids (arginine, histidine and lysine); alkaloids (nicotine and quinine); vitamins (thiamine); and miscellaneous bases such as adenine, pyridine, hydrazine and ammonia.

Basic amino acids separations with AMBERLITE IRC50

With AMBERLITE IRC50, the difficulties in the ion exchange process for basic amino acid separation can be eliminated. Treatment of the carboxylic acid exchanger with an appropriate buffer (sodium acetate-acetic acid) converts the resin to a combined salt-acid form so that cation exchange occurs at a controlled pH. If the ratio of sodium (salt form) to hydrogen (free-acid form) in the exchanger is adjusted to give pH below the isoelectric points of arginine, Hystidine and lysine, but above the isoelectric points of the neutral and acidic amino acids, only the basic amino will exist as cations in solution and will be adsorbed by AMBERLITE IRC50, while the other amino acids will pass through the resin bed unaffected.

Recovery of miscellaneous substances with AMBERLITE IRC50

The unique properties of AMBERLITE IRC50 have been exploited further by determining the adsorption-elution characteristics of the resin in the recovery of many ionic substances, including quinine, nicotine, thiamine, adenine, pyridine, hydrazine, ammonia, and sodium hydroxide. Both the hydrogen and sodium forms were studied in aqueous and alcohol solutions. The results of these investigations are reported in the following tables.
classified bed.

BASICAMINOACIDADSORPTION

Amino Acid	Column Pre-Treatment	Leakage%	Capacity g/L
Histidine	H-resin	25	very low
Lysine	H-resin	25	very low
Arginine	H-rein	25	very low
Histidine	Column buffered—pH5.75	25	very low
Histidine	Column buffered—pH5.30	10	very low
Histidine	Column buffered—pH5.0	5-10	18
Histidine	Column buffered—pH4.7	2-10	35
Lysine	Column buffered—pH7.0	0-3	120
Lysine	Na-resin	Ca-100	very low
Arginine	Column buffered—pH7.0	0-2	150
Arginine	Na-resin	30	—
Leucine	pH4.70 buffer	100	nil
Glutamic Acid	pH4.70 buffer	100	nil

RECOVERYOFMISCELLANEOUSSUBSTANCES

SubstanceRecovered	AMBERLITEIRC50form	Solvent	Leakage%	Capacity g/L
Quininesulphate	Na	H_2O	2	1320
Nicotine	Na	H_2O	100	—
Nicotine	H	H_2O	0-2	385
Thaiminehydrochloride	Na	H_2O	5-8	53.5
Thaiminehydrochloride	Na	C_2H_5OH	25	—
Adenine sulfate	Na	H_2O	100	—
Adenine	H	H_2O	100	—
Pyridine	H	H_2O	0	14
Hydrazine	H	H_2O	0-2	51.2
Ammonia	H	H_2O	0	78
Sodiumhydroxide	H	C_2H_5OH	0	13

LIMITSOFUSE

Rohm and Haas manufactures special resins for food processing and potable water applications. As governmental regulations vary from country to country, it is recommended that potential users seek advice from their Amberlite representative in order to determine the best resin choice and optimum operating conditions.

MATERIALSAFETYDATASHEETS

Material Safety Data Sheets (MSDS) are available for all Amberlite polymeric adsorbents. These sheets contain pertinent information that you may need to protect your employees and customers against any known health or safety hazards associated with our products.
We recommend that you obtain copies of our MSDS from your local Rohm and Haas technical representative before using our products in your facilities. We also suggest that you contact your suppliers of other materials recommended for use with our products for appropriate health and safety precautions before using them.

All our products are produced in ISO 9002 certified manufacturing facilities.

Rohm and Haas/Ion Exchange Resins - Philadelphia, PA - Tel. (800) RHAMBER - Fax: (215) 537-4157
Rohm and Haas/Ion Exchange Resins - 75579 Paris Cedex 12 - Tel. (33) 1 40025000 - Fax: 1 43452819

WEBSITE: http:// www.rohmhaas.com/ ionexchange

ROHM AND HAAS

Ion exchange resins and polymeric adsorbents, as produced, contain by-products resulting from the manufacturing process. The user must determine the extent to which organic by-products must be removed for any particular use and establish techniques to assure that the appropriate level of purity is achieved for that use. The user must ensure compliance with all prudent safety standards and regulatory requirements governing the application. Except where specifically otherwise stated, Rohm and Haas Company does not recommend its ion exchange resins or polymeric adsorbents, as supplied, as being suitable or appropriately pure for any particular use. Consult your Rohm and Haas technical representative for further information. Acidic and basic regenerant solutions are corrosive and should be handled in a manner that will prevent eye and skin contact. Nitric acid and other strong oxidising agents can cause explosive type reactions when mixed with Ion Exchange resins. Proper design of process equipment to prevent rapid build up of pressure is necessary if use of an oxidising agent such as nitric acid is contemplated. Before using strong oxidising agents in contact with Ion Exchange Resins, consult sources knowledgeable in the handling of these materials.